

Protection of Mice Cardiac Myocytes by Polysaccharides from *Cipangopaludina chinensis*

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Abstract

In the present study, we investigated the preliminary characterization, *in vitro* antioxidant and *in vivo* cardiac protective activities of polysaccharides from *Cipangopaludina chinensis* (CCPS). The results for gas chromatography in chemical analysis indicated that CCPS was largely made of glucose with uronic acid and sulfate. *In vitro* antioxidant activity, CCPS demonstrated inhibition effect on lipid peroxidation and Fe²⁺-chelating activity and the hydroxyl radical (\cdot OH) scavenging. For cardiac protective activity *in vivo*, the administration of CCPS significantly decreased the serum levels of alanine aminotransferase and aspartate aminotransferase, inhibited the formation of malondialdehyde in heart and tumor necrosis factor-alpha (TNF- α) in serum and restored the heart activities of superoxide dismutase, glutathione peroxidase in BCG/LPS-induced immunological heart injury mice. The results suggested that CCPS had a significant protective effect against BCG/LPS-induced immunological heart injury. The cardiovascular protective effects of CCPS may be partly because of its immune regulatory activity by suppressing TNF α and antioxidant activity to protect biological system against the oxidative stress response, which they were dependent on the chemical and structural properties of CCPS. The further work on structure for CCPS is in process.

Keywords: Cardiac Myocytes; Polysaccharides; *Cipangopaludina chinensis*

Introduction

Heart failure represents surrogate clinical endpoint for various cardiovascular diseases, promoting to sudden cardiac death. Chronic heart failure is also regarded as a state of chronic inflammation and stress in cardiac tissue. There is strong evidence that reactive oxygen species (ROS) leads to adverse left myocardial remodeling resulting in cardiac contractile dysfunction and finally chronic heart failure [1,2]. Oxidative stress is created by the imbalance between ROS production and its elimination by antioxidant systems. Due to the heart's higher metabolic total rate and controlled regenerative capacity, it is specially sensitive to oxidative damage. On exposure to ROS, the heart involves cell enlargement, myofibrillar disarray, and leads to progressive heart failure [3]. Therefore, some natural products with antioxidant and free radical scavenging activities have attracted great attention as potential functional ingredients to protect cardiomyocyte injuries [4]. Polysaccharides, important natural compounds widely existed in plants, animals and microorganism, have been demonstrated to possess potent antioxidant activity and to protect cardiomyocyte injury induced by various chemicals.

Cipangopaludina chinensis (*C. chinensis*) is widely used in China and many countries. This species resides in pools, lakes, streams and other water bodies and lives on organic particles and microbes [5-7]. Its fresh meat contains high amounts of protein, essential amino acids, taurine, calcium, iron and zinc and is traditionally used as food supplement in china [8,9]. *C. chinensis* has been refined to many different types of food [10,11]. In addition, *C. chinensis* have a long history as a traditional medicine for the treatment of cardiomyocyte injury in China [12]. The studies have indicated that extraction of *C. chinensis* meat exert various pharmacological effects, such as anticancer

activities and protecting cardiac muscle cells against injuries [13,14]. However, little attention was paid to the extracts and multi biological functions of *C. chinensis* polysaccharides. Therefore, the objectives of this study were to determine the *in vitro* antioxidant activities of CCPS and to evaluate its cardiac protective effect on immunological cardiomyocyte injury in mice. Moreover, CCPS was characterised by quantitative chemical analysis, high-quality gas chromatography (GC) and application of Fourier transform-infrared (FT-IR) microspectroscopy.

Materials and Methods

Materials

C. chinensis was obtained from Agricultural and Aquatic Product Market (Huaian, China). Arabinose, fucose, galactose, glucose, mannose, rhamnose, xylose, glucuronic acid and Lipopolysaccharide (LPS) were obtained from Sigma Chemical Co., Ltd (St. Louis, Missouri, USA). Bacillus Calmette-Guérin (BCG) was purchased from the Shanghai Biological Products Research Institute Co., Ltd. in China. Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Ji-an Cheng Bioengineering Institute (Nanjing, China). An enzyme-linked immunosorbent (ELISA) quantitation assay kit for rat tumor necrosis factor- α (TNF- α) was obtained from Shanghai Senxiong Biotech Industry in China. Male Kunming mice were obtained from Guangzhou University of Chinese Medicine in China. All other chemicals and reagents used were of analytical grade.

Preparation of CCPS

The crude CCPS was prepared according to the reported method with some modifications [15]. After removing the shells and impurities, the flesh was crushed by a high speed disintegrator and the homogenate was kept in 90% of ethanol (v/v) for two weeks. Then, the collected flesh was air-dried at 50°C and extracted with distilled water in a ratio of 1:25 (raw material to water, w/v) for 3h at 90°C for three times. After treatment, the mixture was centrifuged at 5000 rpm for 20 minutes, and the insoluble residue was treated again as mentioned above. The supernatants were collected, concentrated to a proper volume by using a vacuum rotary evaporator, deproteinized by the method of [16] and mixed with three times volume of absolute ethanol. The reaction mixture was stirred strongly and kept at 4°C overnight. The precipitant was collected by the centrifugation at 5500 rpm for 25 minutes and air drying at 55°C to constant weight, affording water-soluble crude CCPS.

Preliminary characterization of CCPS

Determination of contents of total sugars, sulfate, protein and uronic acid

The total sugar content of CCPS was determined using the phenol-sulfuric acid method [17]. Protein content was evaluated by Bradford method with bovine serum albumin standard [18]. The uronic acid content was tested [19] using D-glucuronic acid as the standard. The content of sulfate radical was evaluated by the analysis method [20].

FT-IR spectral analysis

FT-IR spectra of CCPS was determined by Thermo Scientific Nicolet 6700 FT-IR Spectrometer with KBr disks. In summary, all samples were then dried at 45°C under vacuum over P₂O₅ for 47h, ground powder and pressed pellet for FT-IR spectra data evaluation in the range of 4000 - 400 cm⁻¹.

Analysis of monosaccharide composition of CCPS

The monosaccharide composition of crude CCPS was determined using the method reported by [21] with slight modification. In summary, the polysaccharide (5.0 mg) was hydrolyzed by 2 M trifluoroacetic acid (TFA 4 ml) in an oven for 1.5h at 110°C, and the extra TFA was evicted with evaporation at the temperature of 45°C. The hydrolyzate was concentrated with methanol dry and acetylated by mixture of methanol, pyridine as well as acetic anhydride. The difference standards of monosaccharide were acetylated in exactly the same way. Finally, the acetylated samples were analyzed by a 7890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization

detector and a HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 mm). The oven temperature was maintained at 120°C for 3 minutes, and then increased gradually to 210°C at a rate of 3°C/min. The detector and injector temperatures were 280°C and 250°C, severally. The flow rates of N₂, H₂ and air were 25, 30 and 400 ml/min, respectively.

Determination of antioxidant activity *in vitro* of CCPS

Assay of Fe²⁺ chelating activity

The Fe²⁺ chelating activity of CCPS was determined according to the reported method [22]. Polysaccharide of sample (1 mL) at concentration range (0, 0.4, 0.8, 1.6, 3.2 and 4.0 mg/ml) was added to 0.05 ml of ferrous chloride (FeCl₂) (2 mM), ferrozine (0.2 mL, 5 mM) and 2.75 ml water. The reaction mixture was shaken vigorously and incubated at room temperature for 15 min, and the absorbance was tested at 600 nm. The Fe²⁺ chelating activity was calculated by the following formula:

$$\text{Fe}^{2+} \text{ chelating activity (\%)} = 100 \times (A_0 - A_1 + A_2) / A_0$$

where A₀ is the absorbance of control sample (water instead of sample), A₁ is the absorbance in the presence of tested sample, and A₂ is the absorbance of the sample only (water instead of FeCl₂ solution). EDTA 2Na (Ethylenediaminetetraacetic acid disodium) served as a positive control in the present work.

Assay of lipid peroxidation inhibition effect

The lipid peroxidation inhibition effect of CCPS was determined by thiobarbituric acid-reactive-substances (TBARS) assay using mouse heart homogenate as the lipid rich media with some modification [23]. Briefly, 1.0 ml of 1% (w/v) mouse heart homogenate was mixed with 1.0 ml sample of the solution with concentrations range (0, 0.4, 0.8, 1.6, 3.2 and 4.0 mg/ml), and 0.05 ml of 0.5 mM FeCl₂ and 0.5 mM H₂O₂ was added to initiate lipid peroxidation, which was carried out in a 37°C water bath for 30 minutes. The reaction was tested by adding 1.5 ml trichloroacetic acid (TCA) and 1.5 ml thiobarbituric acid. The resulting mixture was vortexed and heated in a boiling water bath for 10 minutes. After being centrifuged at 4000 rpm for 15 minutes, the TCA-TBA phase was removed entirely and the absorbance was measured at 535 nm. Butylated hydroxytoluene (BHT) served as a positive control. The inhibition effect on lipid peroxidation was calculated according to the formula below:

$$\text{Lipid peroxidation inhibition effect (\%)} = 100 \times (A_0 - A_1 + A_2) / A_0$$

where A₀ is the absorbance of control sample (water instead of sample), A₁ is the absorbance in the presence of tested sample, and A₂ is the absorbance of the sample only (water instead of heart homogenate).

Assay of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by the method of [24] with some modifications. The hydroxyl radical (.OH) was produced by the mixture of 1 ml of 1,10-phenanthroline monohydrate (0.75 mM), 1.5 ml of sodium phosphate buffer solution (0.15 M, pH 7.4), 1 ml of FeSO₄ (0.75 mM) and 1 ml 0.01% H₂O₂ v/v. After adding 1 ml sample, the resultant mixture was incubated for 30 minutes at 38°C. The absorbance of the reaction mixture was calculated at 535 nm. The hydroxyl radical scavenging activity was calculated by the following formula:

$$\text{Hydroxyl radical scavenging activity (\%)} = 100 \times (A_1 - A_0) / A_2 - A_0$$

where A₀ is the absorbance of control sample (water instead of sample), A₁ is the absorbance in the presence of tested samples, and A₂ is the absorbance of water instead of H₂O₂ and sample in the assay system. Ascorbic acid served as a positive control.

Evaluation of cardioprotective effects of CCPS

Animal grouping and experimental design

The cardiac protective effects of CCPS on immunological heart injury in mice were evaluated. In summary, Kunming male mice (20 ± 2g, 8 weeks old) were selected in this study. The animals were maintained in standard environmental conditions (22 ± 0.5°C 55 ± 5%

humidity and a 12h light/12h dark cycle) and maintained with free access to standard laboratory pellet diet and water. The scientific procedures involving animals were in strict accordance to the People's Republic Chinese legislation. After a one-week acclimatization period, the mice were randomly assigned to six groups (n = 10 for each group) including normal control, model control, silymarin positive control and CCPS groups. On the first day of the experiment, BCG (2.5 mg, suspended in 0.2 ml saline) was injected into the tail vein of each mouse, except for the normal control group, which accepted saline alone. The mice in CCPS groups were given CCPS in three doses (200, 400 and 600 mg/kg BW/day, separately) by gastrogavage. The mice in the positive control group were fed silymarin at 50 mg/kg BW/day for 15 days before the LPS injection. Normal and model groups accepted the same volume of physiological saline solution.

Biochemical assay

Then after 8 hours the CCPS-treated or silymarin-treated, the LPS+BCG-induced mice, silymarin treatment and CCPS treatment groups were treated by LPS (7.5 µg /each mouse) via the tail vein injections. Mice were then fasted for 16h before they were sacrificed. Blood samples were collected immediately and centrifuged at 3000 rpm at 4°C for 10 minutes to afford the serums. The heart was excised and homogenized immediately in 0.1 g/ml wet weight of ice-cold physiological saline solution. The top suspension was then separated and the supernatant liquid was extracted for the further analysis. All above treatments were done at 4°C.

The activities of SOD and GSH-Px, MDA and TNF-a levels were measured by commercially available assay kits in accordance with the manufacturer's instructions. In summary, the protein content was determined with the method of Bradford using bovine serum albumin standard. The SOD and GSH-Px activities were measured in accordance with the reaction system of xanthine and oxidase-xanthine and decreased glutathione (GSH)-H₂O₂ reaction system, severally. These enzyme activities were expressed as U/mg of protein. MDA level was measured by the TBARS method and expressed as nmol per milligram of protein (nmol/mg protein). In addition, serum TNF-a was determined by ELISA according the manufacturer's protocol and expressed in nanograms per milliliter (ng/ml).

Histopathological evaluation

Histopathological analysis of the heart tissue was mixed in 10% buffered formalin, the tissue was treated with routine histology and imbedded in the paraffin. Sections of tissue (4 - 5 µm) were examined by hematoxylin and eosin staining and collected for histopathological changes under high resolution digital microscope with digital photography facility.

Statistical analysis

Data were reported as mean ± SD (standard deviation) and determined by One-way ANOVA offered by Duncan's tests. The p-values of less than 0.05 were considered as significant. The statistical analyses were performed using SPSS statistics, version 13.0 software (SPSS Inc, Chicago, IL).

Results

Preliminary characterization of CCPS

The CCPS was firstly isolated from *C. chinensis* by the hot water extract, ethanol precipitation and the vacuum freeze-drying. An overall yield of CCPS was 8.7% based on the dried fresh used. Results of the chemical analysis demonstrated that the neutral sugar contents and protein of CCPS were measured to be 78.4% and 5.1%, severally. CCPS included the high contents of 6.3% uronic acid and 7.7% sulfate. The monosaccharide composition of CCPS was determined by GC, and the monosaccharides in CCPS hydrolyte were identified by comparing the retention times with those of standards. As shown, CCPS was mainly composed of glucose (95.2%) together with 4.2% rhamnose and 0.6% fucose.

The FT-IR spectrum analysis of CCPS demonstrated that a broad and high absorption peak at 3431 cm⁻¹ for the O-H stretching vibrations, a peak at 2928 cm⁻¹ for the C-H stretching vibrations, and a strong extensive absorption in the region of 900 - 1200 cm⁻¹ for coupled C-O and C-C stretching and C-OH bending vibrations were observed in CCPS, indicating the characteristic absorptions of polysaccharides [25]. The strong peak at 1239 cm⁻¹ due to the stretching vibration of the S-O, showed that CCPS contained the sulfate group. The bands in

the region of 1661, 1634 and 1443 cm^{-1} , characteristic of the carboxylic group, showed the presence of uronic acid in the polysaccharides. The results were in accordance with the analytic results of sulfate and uronic acid contents in CCPS.

Antioxidant activities *in vitro* of CCPS

Fe²⁺ chelating activity of CCPS

The Fe²⁺ chelating activities of CCPS and EDTA-2Na showed the Fe²⁺ chelating activity of CCPS was evident at all of the tested concentrations. In addition, the Fe²⁺ chelating activity was correlated well with the increase of concentration up to 4.0 mg/ml. At a concentration of 4.0 mg/ml, the Fe²⁺ chelating activity for CCPS was 83.2%. The Fe²⁺ chelating activity of CCPS was similar to that of EDTA-2Na at 4.0 mg/ml. The results demonstrated that CCPS possessed strong Fe²⁺ chelating activity.

Lipid peroxidation inhibition effect of CCPS

The inhibitory effects on lipid peroxidation of CCPS and BHT elevated with the elevation of sample concentration. At a concentration of 4.0 mg/ml, the inhibitory rate of CCPS on lipid peroxidation was 65.7%, which was lower than that of BHT. The result suggested that CCPS possessed medium moderate lipid peroxidation inhibition activity.

Hydroxyl radical scavenging activity of CCPS

Scavenging effects of hydroxyl radical in CCPS and vitamin C were elevated with the elevation of concentration (4.0 mg/ml). At a concentration (4.0 mg/ml), the hydroxyl radical scavenging activity was 82.1%, which was close to that of ascorbic acid. The results showed that CCPS had high scavenging activity of hydroxyl radical.

Cardioprotective effects of CCPS

Effects of CCPS on heart SOD, GSH-Px and MDA in BCG/LPS injured mice

The MDA levels and activities of SOD and GSH-Px in heart are shown in table 1. Compared with those of normal control group, there was a significant increase in the MDA level and decrease in the activities of SOD and GSH-Px in BCG/LPS model group ($P < 0.05$). The results demonstrated that the experimental model was successfully made in this study. CCPS remarkably elevated the activities of SOD and GSH-Px ($P < 0.05$), whereas diminished MDA level as compared with those of BCG/LPS-induced group.

Group	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)
Positive control group	196 ± 41	445 ± 56	1.6 ± 0.25
Model control group	119 ± 20	281 ± 47	3.8 ± 0.23
Normal control group	218 ± 32	536 ± 72	1.1 ± 0.17
P value	< 0.05	< 0.05	< 0.05
Model control group	119 ± 20	281 ± 47	3.8 ± 0.23
CCPS treatment group, 200 mg/kg	121 ± 34	310 ± 69	3.5 ± 0.28
P value	< 0.05	< 0.05	< 0.05
Model control group	119 ± 20	281 ± 47	3.8 ± 0.23
CCPS treatment group, 400 mg/kg	123 ± 26	339 ± 87	2.9 ± 0.33
P value	< 0.05	< 0.05	< 0.05
Model control group	119 ± 20	281 ± 47	3.8 ± 0.23
CCPS treatment group, 600 mg/kg	157 ± 23	345 ± 74	2.1 ± 0.29
P value	< 0.05	< 0.05	< 0.05

Table 1: Effects of CCPS on heart SOD, GSH-Px and MDA in BCG/LPS-injured mice.

Data were presented as mean ± SD (n=10) by one-way ANOVA followed by the Duncan's multiple-range test.

Effects of CCPS on serum TNF-a in BCG/LPS-injured mice

In BCG-primed mice challenged with LPS when compared with those of normal control group. The administration of CCPS at a high dose (600 mg/kg BW) significantly inhibited the production of TNF-a, while CCPS at doses of 200 mg/kg BW or 400 mg/kg BW had no significantly effect on TNF-a level (Table 2).

Group	TNF-a (ng/ml)
Model control group	5.9 ± 2.0
CCPS treatment group, 600 mg/kg	3.1 ± 3.1
P value	< 0.01

Table 2: Effects of CCPS on serum TNF-a in BCG/LPS-injured mice.

Data were presented as mean ± SD (n = 10) by one-way ANOVA followed by the Duncan’s multiple-range tests.

Histopathological results

The effects of CCPS on heart histopathology of BCG/LPS-treated mice showed the heart section from the control group, it showed normal cardiac cells with well-preserved cytoplasm and prominent nucleus, while massive inflammation and infiltration were observed in the heart section of BCG/LPS-treated mice in model control group (Figure 1). However, the administration of CCPS decreased the level of the cardiac lesions especially at a dose of 600 mg/kg BW. The histopathological observations were in agreement with biochemical index measurements and further confirmed the cardiac protective potential of CCPS.

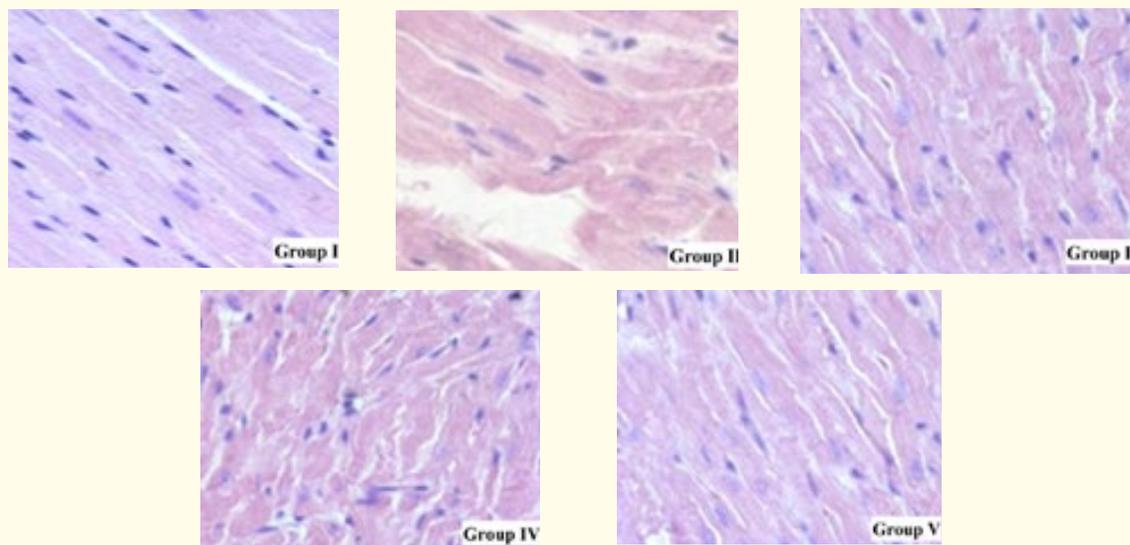


Figure 1: Pathological changes in myocardium tissue (H&E, ×200 magnification, scale bars = 50 μm).

Discussion

Oxidative stress, considered as an excessive production of ROS relative to antioxidant defense system, played a very important role in the cardiac pathophysiology and heart failure progresses but clinical trials of antioxidant approaches to prevent cardiovascular morbidity and mortality have been unsuccessful [26,27]. Therefore, there is a worldwide trend to go back to natural products origin that are in use for the treatment of heart ailments [28]. The study demonstrated that polysaccharides from *Apostichopus japonicus* had important

scavenging activities of free radical and had a protective effect on hyperlipidemia [29]. It has been demonstrated that *C. chinensis* has a history as a traditional herbal medicine for the treatment of heart diseases [30].

Whereas cardiac protective effect in CCPS is not very clear. In the present study, we investigated the cardiac protective effects of CCPS on heart injury in mice. It is recognized that immune factors is the predominant reasons of heart injury. The model of immunological heart injury induced by BCG plus LPS is similar to the pathological course of chronic heart diseases in humans [31-33]. It is known that BCG priming and LPS challenge in mice causes massive heart injury, which consists of priming and eliciting phases. BCG induced mononuclear cell infiltrates into the aortic endothelial cells. The subsequent LPS injection elicits heart injury, with a host release of reactive oxygen species, nitric oxide [34]. Silymarin, an antioxidant flavonoid complex derived from milk thistle (*Silybum marianum*), has cardioprotective activity against ischemia-reperfusion-induced myocardial infarction in rats [35].

In this study, therefore, mice treated with BCG/LPS were used as cardiac toxicity animal model and silymarin was used as positive control medicine. The results indicated that CCPS had cardiac protective effect against BCG/LPS-induced heart injury in mice.

Oxidative stress is considered as a very important role in the BCG plus LPS-induced immune injury to cardiac model. It is defined as a process in which the balance between prooxidants and antioxidants is shifted toward the oxidant side. ROS molecules, one of prooxidants, are chemically reactive oxygen metabolites. Physiologic conditions to preserve the physiological level of ROS molecules are important for the regulations of cellular functions such as cellular signaling pathways, activation of transcription, cellular hyperplasia, inflammation and apoptosis. The cellular activity of antioxidant enzymes plays important roles in the protection of biological systems against the oxidative stress damage by cleaning ROS molecules and preserving the balance between production and elimination of ROS. However, once organisms are exposed to some exogenous and endogenous factors, the balance between generation and removal of ROS will be broken. The resulting excessive accumulation of ROS causes lipid peroxidation by attacking the polyunsaturated fatty acids in the bio-membranes, and subsequently results in detriment of membranes and its function, injury of tissues and organs, even leads to some diseases and aging.

To confirm the effect of CCPS on oxidative stress in immunological heart injury, we examined the oxidative stress parameters, including SOD, GSH-Px and MDA. The present study demonstrated that the antioxidant enzymes (SOD and GSH-Px) were remarkably reduced and the MDA level was remarkably elevated in BCG/LPS-treated mice as compared to those of normal group. We suggested that BCG/LPS treatment remarkably reduced antioxidant activities in BCG/LPS-induced cardiac damage in mice and promoted the free radical accumulation which subsequently led to increase level of lipid peroxidation. However, CCPS administration resulted in the reduction of MDA level and increase of SOD and GSH-Px activities in BCG/LPS-induced heart injury mice. In addition, we determined the antioxidant activities *in vitro* of CCPS and found that CCPS exhibited medium lipid peroxidation inhibition effect and high Fe²⁺ chelating and hydroxyl radical scavenging activities. Our study intimated that CCPS had an important role in the cardioprotective effect on BCG/LPS-induced heart damage possibly due to its antioxidant activities to suppress oxidative stress.

According to the results stated above and some other literatures, it is believed that the cardiac protective effect of polysaccharide is related to its antioxidant activity, which is dependent on the chemical and monosaccharide composition, molecular weight and function groups of polysaccharides. Polysaccharides with relatively low molecular weight and high protein content are found to possess high antioxidant activity [36]. The high uronic acid and sulfate content were found to be beneficial to the antioxidant activities of the polysaccharide [37,38]. In the present study, we found that CCPS had high contents of uronic acid and sulfate. Our results suggested that the chemical and structural properties of CCPS might contribute to its moderate lipid peroxidation inhibition effect and strong Fe²⁺ chelating and hydroxyl radical scavenging activities, and then be related to its cardiac protective effect on BCG/LPS-induced heart injury.

TNF- α played an important part in immune-mediated cardiac myocyte damage and promoted progression of congestive heart failure. TNF- α increases oxidative stress and apoptosis in cardiomyocytes by upregulating p38 mitogen-activated protein kinase phosphorylation [39,40]. Our results demonstrated that BCG/LPS treatment led to significant increase of serum TNF- α level in mice, indicating consider-

able inflammatory response. On the other site, the administration of CCPS markedly decreased the level of serum TNF- α compared to the model control group. The study indicated that CCPS inhibited production of TNF- α and BCG plus LPS-induced cardiac injury in mice.

Conclusion

In the present study, CCPS was preliminarily characterized to be composed of 95.2% glucose, rhamnose (4.2%) and fucose (0.6%). The assay of the antioxidant activities *in vitro* demonstrated that CCPS had medium lipid peroxidation inhibition effect and high Fe²⁺ chelating and hydroxyl radical scavenging activities. The study of myocardial protective activity *in vivo* showed that the CCPS remarkably reduced serum TNF- α and MDA levels and significantly reinstated the heart activities of SOD and GSH-Px in BCG/LPS-induced immunological heart injury mice. The study showed that CCPS had a significant myocardial protective effect on BCG plus LPS-induced immune cardiac injury in mice. The cardiac protective effect of CCPS might be partly due to the immunoregulatory effect by inhibiting TNF- α production and antioxidant activity to protect biological systems against the oxidative stress, which is dependent on the chemical and structural properties of CCPS. The further study on CCPS structure is now in progress.

Competing Interests Statement

The authors declared that no competing financial interests.

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